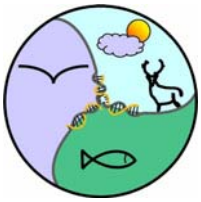
Early development of grayling in Lake Lesjaskogvatn: a reciprocal transplant field experiment

Eirik Krogstad

Master of Science thesis



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Oslo, February 2008

Early development of grayling in Lake Lesjaskogvatn: a reciprocal transplant field experiment

Master thesis – Eirik Krogstad

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Abstract

Separate spawning demes of grayling have established during the course of 20-25 generations in the Norwegian mountain lake Lesjaskogsvatn, where the spawning streams have consistently differing temperature regimes. Recent studies have indicated countergradient growth adaptation in the early life stages for demes inhabiting cold streams, possibly due to time constraint from a short growth season and size-dependent winter mortality. Several crosses of artificially fertilized grayling eggs were employed in a reciprocal transplant field experiment between tributaries with differing temperature patterns. The data was analysed for presence of genetic variation, maternal effects, and local adaptation in growth and other ontogenetic properties between cold and warm demes. Additive genetic effects on growth, differences in mean egg size, and maternal effects on size were found. There were differences in size and growth rate between cold and warm demes and across environments. Reaction norms showed indications of genotype x environment interactions, although for the most part inconsistent with signatures of local adaptation. Large variation from environmental effects remains unaccounted for. The findings do not confirm the presence of countergradient variation, but additive genetic variance shows there is capacity for adaptation, and maternal effects include the possibility for an adaptive increase in egg size.

Introduction

A number of recent studies document high rates of microevolution over short periods of time (Thompson 1998; Hendry and Kinnison 1999), making adaptive change observable on ecological time scales. The conditions that promote contemporary adaptation are frequently one of two settings: colonization events, in which colonists are isolated from their ancestral population and adapt to a new environment, and local adaptation in heterogenous environments with a metapopulation structure (Reznick and Ghalambor 2001). In contrast to adaptive colonization events, genetic differentiation can also occur if the colonizing population is small. This constitutes a bottleneck, through which the genetic variation of the colonists is unlikely to be representative of the source population. Random genetic drift is likely to occur, further altering allele frequencies and reducing variation compared to the source population (eg. Futuyma 2005). These two modes of differentiation are driven by non-random (selection) and random (genetic drift) forces, respectively.

The Norwegian mountain lake Lesjaskogsvatn and its neighbouring lakes have been subject to several life history and evolutionary studies on grayling (*Thymallus thymallus*). The grayling here are remarkable in many ways: they may have been subject to all of the modes of differentiation mentioned above, and show rates of evolution among the highest ever reported for the species (Koskinen et al 2002). The grayling have shown capacity of local adaptation even in the presence of low genetic variation and genetic drift. Haugen and Vøllestad (2000) conducted a comparative study of grayling populations in Lesjaskogsvatn

and nearby lakes colonized from it. They found significant additive genetic variance and differentiation in early life history traits between populations. Egg size explained most of the variation in traits with a maternal effect, including size. The different populations grew best at the temperatures they experienced in nature, suggesting local adaptation. Analyses of microsattelites show signatures of severe bottlenecks, but natural selection has been shown to be the main diversifying agent (Koskinen et al 2002).

Lesjaskogsvatn itself was colonized by grayling some 120 years ago (Haugen and Vøllestad 2001). The founding population is again thought to have been small, as there is evidence of a bottleneck (Koskinen et al 2000). Separately breeding demes have since been established (Gregersen et al 2008), made possible by the strong homing behaviour of the grayling; mature grayling will return with high precision to their natal stream to spawn (Kristiansen and Døving 1996). There is genetic differentiation by distance between most of the demes, and the pattern suggests that genetic structuring is developing, with some gene flow (Barson et al 2008). Kavanagh et al. (2008) have recently presented evidence from a common garden laboratory study that the grayling within Lesjaskogsvatn have evolved a countergradient growth adaptation in the early life stages. The spawning streams in Lesjaskogsvatn have consistent and predictable differences in temperature regime, divided into cold and warm streams due to geographical features. Demes in cold streams show higher embryonic growth rates, higher yolk sac absorption rates and yolk conversion efficiencies than those breeding in warm streams. The time of spawning is consistently later in cold streams, and the temperature total lower. Growth is temperature dependent, leaving cold demes with a severe time constraint though a short sub-arctic growth season.

Winter mortality is a large threat to young-of-the-year (age-0) fish. Thermal stress is likely to be more lethal to small individuals, and starvation a greater risk because of lower available energy reserves combined with decreased or halted feeding. A lot of evidence points towards a positive size-dependence for winter mortality (Hurst 2007). Winter mortality may have acted as a powerful selective force for cold demes in Lesjaskogsvatn, driving an adaptive response in growth that at least partially counteracts the negative effects of temperature and short growing season.

Life history traits such as growth are usually polygenic, and are best measured by their phenotypic expression. This requires the framework of quantitative genetics, as explained by Roff (1997). Through common garden or reciprocal transplant experiments, we can partition the phenotypic variation (V_P) in polygenic traits:

$$V_P = V_G + V_E + V_{GxE} + 2Cov(G,E)$$

In the model above, V_E is variation due to environmental sources, i.e. from phenotypic plasticity. The remaining variation will be a combination of variation from purely genetic sources (V_G), genotype x environment interaction (V_{GxE}) and genotype x environment covariance (Cov(G,E)). V_{GxE} is the extent to which genotypes differ in their response to environmental effects, Cov(G,E) is the extent to which genotypes are non-randomly distributed across a range of environments, including countergradient variation. Countergradient variation is the specific case of genetic-environmental covariance where genotypes are distributed such that genetic and environmental influences oppose one another; when Cov(G,E) is negative (Conover and Schultz 1995). It is possible to detect these effects when we examine the reaction norms of genotypes across environmental gradients.

In a study of early life history traits, it is important to consider the different contributions of parents to their offspring. Paternal contributions in fish are generally purely genetic (Kamler 2005). By comparing half-sibs with the same dam, maternal contributions are kept constant, and paternal genetic contributions can be quantified.

On the other hand, maternal contributions have several components. In fish, egg quality and growth factors may influence offspring, but the energetic value of the egg is the dominant maternal contribution (Kamler 2005). In addition, mothers have a genetic contribution. The maternal contribution to the egg itself may confound the effect of genotype, so these elements should be separated. The total maternal effects may be estimated by comparing full-sibs and half-sibs, both with the same sire. The size of the non-genetic maternal contribution can be then be approximated by measuring the energetic value of the egg for comparison. Dry egg weight is the closest measure of caloric value besides measuring it directly (Kamler 1992).

We performed several artificial crosses on spawning grayling and a reciprocal transplant field experiment between streams with differing temperature patterns in Lesjaskogsvatn. Morphometric data on the early life stages is analysed for genetic (paternal), maternal, and environmental effects and their interactions. We expect some degree of additive genetic variance between demes. If large maternal effects are present, we expect them to have a significant effect on growth. If the countergradient growth adaptation hypothesis and the findings of Kavanagh et al (2008) are correct, then we expect to see a pattern of higher growth rate and higher yolk-sac absorption in cold stream offspring versus those in warm streams.

Materials and methods

The subject organism

The European grayling is a salmonid fish common throughout central, northern and eastern Europe. It is an spring spawner, and usually iteroparous. Spawning typically takes place in streams after ice-break, when the temperature climbs above 4-7°C (Northcote 1995). The grayling have been observed to wait at the stream outlet for the optimal time of spawning, and maturation of gonads likely takes place during this time. Temperature is considered the main trigger for spawning (Fabricius and Gustafson 1955), but stream discharge and turbidity may also have an influence. At spawning, eggs are deposited a couple of centimetres below the gravel surface in the stream bed while being fertilized, and are then left unattended.

Early grayling development and growth is temperature-dependent. Recent laboratory work at the University of Oslo suggests that a constant temperature of 5°C is the lower limit for successful embryonic development, beneath which growth is essentially halted (unpublished results, N. Barson, University of Oslo). This at least seems to be the case for the Lesjaskogsvatn population, although temperatures generally fluctuate more in the wild. Best survival is attained between 6°C and 13.5°C, and there is 100% mortality below 2°C, and above 16°C according to Jungwirth and Winkler (1984). Survival at higher temperatures has been documented (see below). Timing of developmental events is commonly represented in degree-days (D°), defined as the number of days multiplicated by temperature. Mean hatching time for the larvae lies in the range of 170-220 D° (Kokurewicz et al 1980; Scott 1985; Northcote 1995). After hatching, larvae stay in the gravel until the yolk sac is absorbed. At temperatures between 12 and 18°C, this lasts 5-10 days (d'Hulstere and Philippart, 1982), corresponding to 90-120 D°. They then emerge from the gravel as fry. Grayling fry are poor swimmers, but if current velocity is low, they may stay within the stream for some time before travelling downstream (Bardonnet and Gaudin 1990; Northcote 1995).

Lesjaskogsvatnet is a shallow mountain lake in central Norway (62°20'N, 8°40'E, altitude 611 m). The lake drains into two rivers, Rauma in the west and Gudbrandsdalslågen in the east, with tributaries running down from the northern and southern mountainsides. Tributaries on the northern slope get more sunlight, and are generally smaller and warmer than those on the shady southern side throughout the summer (Gregersen 2005). The temperature differences are consistent from year to year, and small, northern tributaries reach the observed lower temperature for spawning earlier than the large cold ones (Kavanagh et al 2008) Lake Lesjaskogsvatn was colonized by grayling in the early 1880s by a man-made connection to the river Gudbrandsdalslågen. Removal of this connection shortly after has made further upstream migration difficult, but grayling may migrate downstream to the river (Haugen and Vøllestad 2001). After some 20-25 generations, at least 20 demes are currently found in the lake (Gregersen et al 2008). The adults live sympatrically in the lake, but spawn in different tributaries.

In previous studies of Lesjaskogsvatn and nearby lakes colonized from it, evidence has been found for adaptive divergence over the course of 9-25 generations. In 1910, grayling were transported from Lesjaskogsvatn to the nearby lake Hårrtjønn, and this population migrated downstream to Aursjøen in the 1920s. These lakes are subject to the same macro-environmental conditions; however, local environmental conditions vary (Haugen and Vøllestad 2001). Haugen and Vøllestad (2000) found significant additive genetic variance in length and yolk-sac volume, and significantly different reaction norms for growth rate and survival during the period of first feeding. This was in a common garden laboratory study comparing the Lesjaskogsvatn, Hårrtjønn and Aursjøen populations. Using the same data, these traits were shown to have high evolution and divergence rates compared to other life-history studies on the same temporal scale (Haugen and Vøllestad 2001). Recent work on the Lesjaskogsvatn system (Kavanagh et al 2008), comparing early development in cold and warm demes, have shown higher embryonic growth rate, yolk-sac absorption rate and yolk sac conversion efficiency in cold demes. Growth rate differences are maintained for several weeks. Cold demes also had accelerated muscle and skeletal development, but no external morphometric differences were found.

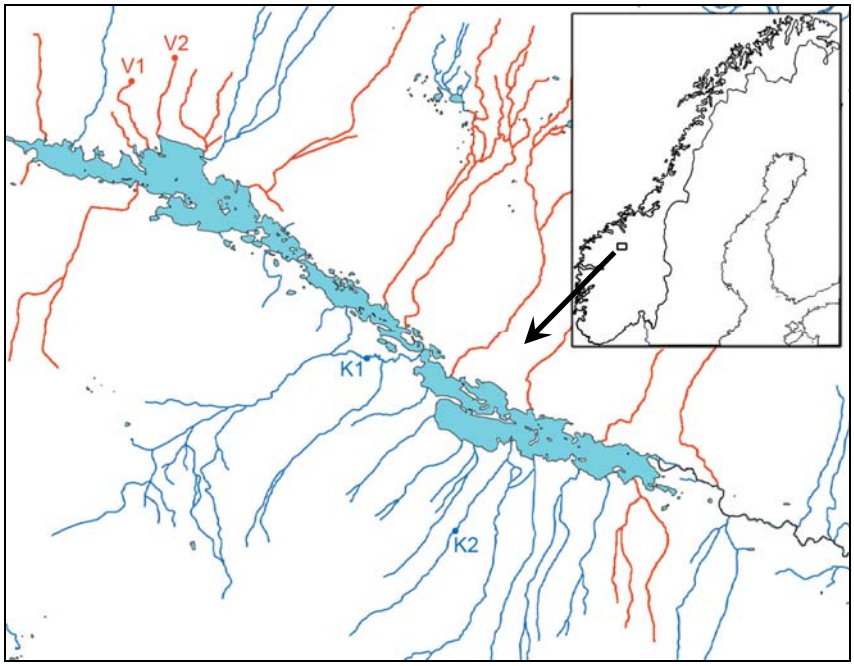


Figure 1. Map of Lake Lesjaskogsvatn.

Tributaries fall into two groups, those with cold mean temperatures in summer (shown in blue) and those with warm mean temperatures (red). V1 and V2 are the warm temperature streams used in the experiment, K1 and K2 are the two cold ones.
Gudbrandsdalslågen is the river marked in black in the eastern end of the lake.

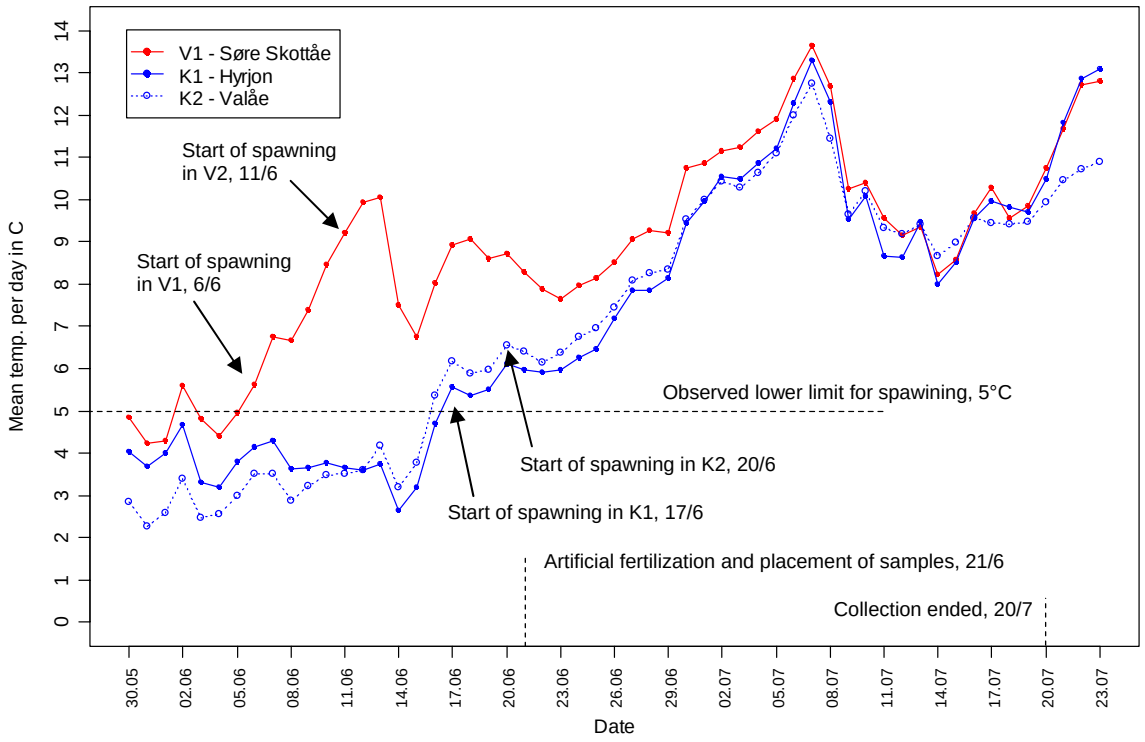


Figure 2. Temperature development in lake Lesjaskogsvatn tributaries in 2006, based on diel means from temperature logger measurements every 2 hours. Start of spawning is noted for the four tributaries. Temperatures were not available for the V2 tributary, see details in the text. The period in which the reciprocal transplant experiment took place is also marked.

Field experiment

A field experiment was performed at Lake Lesjaskogsvatn during the summer of 2006. Four known spawning tributaries (Gregersen 2005) to the lake were selected for their temperature properties, see figure 1. Two were selected as warm tributaries, two as cold. The warm tributaries were Søre Skottåe and Steinbekken, the cold were the eastern arms of Hyrjon and Valåe. These were designated V1, V2, K1 and K2, respectively. Temperature was measured throughout the period using HOBO® loggers. We did not have a temperature logger in the V2 stream in 2006, but this stream showed high correlation with V1 in 2005 (r=0.98, n=8) and in 2007 (r=0.98, n=109). V1 and V2 temperature curves followed each other very closely in 2007 (figure 14, appendix). Manual measurements with a mercury thermometer in 2005 and 2006 confirm this, and the two streams are located about 500 m apart. For the purposes of analysis here, V1 temperature data were used for V2. The temperature development, times of spawning and sampling period are described in figure 2.

The streams were fitted with traps prior to spawning in the two warm tributaries, and at the start of spawning in the cold ones, as high flow rates made secure placement of the traps difficult. The traps covered the entire width of the stream, so all fish going upstream to spawn would be captured. We tried to place the traps at locations downstream from areas where fish were likely to spawn, but some spawning may have taken place below this point. Fish were usually captured in the traps during the night. They were moved as soon as possible to tanks submerged in the stream, with good water ventilation. They were then taken out at random the same day they were found in the traps, sedated using a fairly standard dosage of 50-150 mg/L Benzocaine (ethyl aminobenzoate) (Schreck and Moyle 1990), and their weight and length were measured. Among the first spawners captured, 15 males and 15 females from each stream were stripped of milt and eggs. The gametes were

conserved in closed plastic bags with ample air, and then stored in cool containers (4-8°C). Care was taken not to introduce moisture, as this could shorten their lifespan. Eggs start swelling when exposed to water, and this lowers their fertility. Sperm motility is suppressed by high K^+ concentration in the milt, and dilution in water activates the sperm. Activation would quickly deplete the energy of sperm cells, making them unable to fertilize the egg. When stored correctly *in vitro,* eggs survive only a few days, while sperm may survive a few weeks (Billard et al 1986; Schreck and Moyle 1990). Separate egg samples were taken for egg weight measurements, and frozen on site. Spawning took place in the last cold stream 14 days after the first warm one. Because we feared for the quality of the first batch of gametes, new material was taken from V1 (a new set of 15 samples per sex) and V2 (14 samples from females) close to the time of spawning in the cold streams. These were the ones used for artificial fertilization and weight measurement.

The experiment was set up in the form of a partial reciprocal transplant, with two pairs of cold and warm tributaries. The milt from each stream was pooled, and introduced separately to the eggs of each single dam according the crossing scheme, see table 1 and below. In this way, the sperm from each sire had the opportunity to fertilize the eggs of each dam, as long as the sperm was healthy. The eggs were then pooled for each of the 12 cross types. The process of activating fertilization is described by Haugen (2000a).

Table 1. Cross scheme for the experiment. P and/or M denotes placement of fertilized eggs of corresponding cross type in the tributary. Fields marked P in the same stream allow contrasting parental effects. Fields marked M allows assessment of maternal effects. Cold endemics were placed in both their native stream and a warm stream, and warm endemics were placed both in their native stream and a cold stream, as marked by the grey background. The other cross types were exclusively placed in one stream.

	Stream			
Cross-type	**K1**	**V1**	**K2**	**V2**
K1 females x K1 males	P	M		
K2 females x K2 males			P	M
K1 females x K2 males	P			
K2 females x K1 males			P	
K1 females x V1 males	P/M			
K2 females x V2 males			P/M	
V1 females x K1 males		P/M		
V2 females x K2 males				P/M
V1 females x V2 males		P		
V2 females x V1 males				P
V1 females x V1 males	M	P		
V2 females x V2 males			M	P

The various cross types have different analytical uses. Pure-genotype crosses, transplanted between tributaries, were used for assessment of environmental effects and genotype x environment interactions. K1xK1 and K2xK2 are in this text referred to as *cold endemics*, V1xV1 and V2xV2 as *warm endemics* (specific cross types are consistently written as *dam x sire*). They were placed in both their native stream, and the paired stream of opposite temperature regime. The eight hybrid cross types (K1xK2, K2xK1, V1xV2, V2xV1, K1xV1, K2xV2, V1xK1, V2xK2) were exclusive to each stream, and could be used to assess additive genetic effects, by comparing cross types with the same dam and sires from different streams (*same-dam*), within the same stream. We also had the opportunity to quantify maternal effects by comparing cross types with the same sire and dams from different streams (*same-sire*), in the same stream (although not the sire's native stream).

30-50 fertilized eggs from each batch according to the cross scheme were placed in small mesh bags, together with fine gravel from the location. 25 bags from each cross type were placed in the gravel of spawning grounds in the streams, over a period of 10 hours.

4 days after fertilization, we started collecting the bags, one of each cross type per stream per day. This was done every day, with some exceptions. Collection of the bags from the various streams was done within a period of 8 hours, and seldom more than 4 hours. The four bags that constituted the daily sample for each cross type were bound together, so variation due to placement within the stream is similar between cross types sampled on the same day. Embryos, and later hatched larvae, were immediately conserved in a 10% buffered formalin solution. Buffered formalin was chosen because it gives little morphic warping, and preserves calcified structures for later measurements (Schreck and Moyle 1990). There was some variation in survival of eggs among different streams and cross types. Eggs in the V1 stream were extensively attacked by fungi, though whether this was due to low gamete quality from storage, low fertilization success, high mortality, conditions in the stream itself, or a combination, is hard to judge Only a few individuals survived. Some fungal growth was also present in the other streams, most notably in V2.

Data acquisition

We wanted to preserve the sampled material for later examination. Due to the fragility of the embryos and larvae, the samples were placed under a Leica® MZ8 microscope fitted with a Leica® DC300 digital camera, and pictures were taken for measurements. A microscope scale slide was also photographed at each magnification. Embryos were carefully dissected out of their shells. Length, taken from the tip of the snout to the visible end of the notochord, yolk sac height *or* width and length (with length always parallel to the axis of the body, and height or width perpendicular to length) and eye diameter were measured using UTHSCSA ImageTool (available at http://ddsdx.uthscsa.edu/dig/itdesc.html). A segmented line is drawn along the axis of measurement, and the program calculates total length, with the scale slide used for scale; see figure 3 for illustration. The pictures were taken at an angle appropriate for the measurements, and perspective shortening was for the most part avoided. See box 1 for estimates of measurement error. It was also noted whether the individual had hatched. Volume of the yolk sac was calculated from its length and height/width, assuming the shape to be that of a prolate spheroid ($4/3\pi ab^2$, with a always set as the longest of the two). The time extent of the data set is from 10 to 28 days after fertilization. Degree-days (D°) were calculated from the mean of 12 daily measurements (every two hours), summed cumulatively from the time of placement of fertilized eggs in the streams. Pre-fertilization egg samples were dried for 5 days at 60°C and eggs from each female were weighed on a Mettler AE 160 weight, with a precision of 0.1 mg. A few of the egg bags were destroyed in storage, the number of replicates were 11 females for K1 and K2, 14 for V1 and 15 for V2, 10 eggs each.

Box 1.

3 measurements were taken of each trait, on each of 10 individuals to estimate the measurement error. Mean error was calculated from the error percentage of each set of 3 measurements: $(\sigma / \bar{x}) \times 100$

The highest error percentage obtained is shown as (max).

	Mean error (max)
Body length	0.71 (1.85) %
Eye diameter	1.12 (2.82) %
Yolk sac volume	2.70 (10.5) %

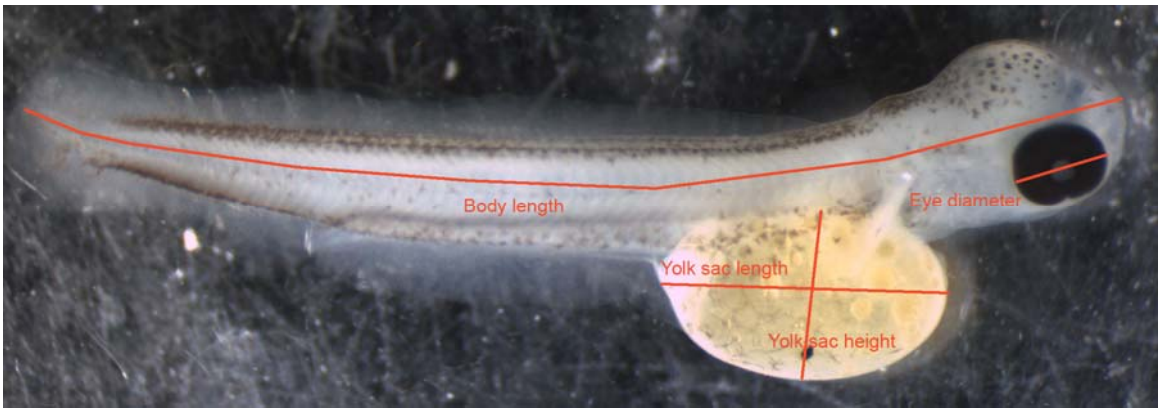


Figure 3. Morphometric measures. Body length was measured from the tip of the snout to the end of the notochord. Eye diameter was measured at the widest point. Yolk sac was measured as length along the axis of the body, and height perpendicular to length. This particular individual is a K2xK2 cross collected from V2 at 16 DPF, corresponding to 174 D°.

Analysis

Analysis of the data was done using R v2.6.1 (available at http://cran.r-project.org/). General linear models (GLMs) were fitted for body length and eye diameter, in each of several comparative levels: between same-dam or same-sire cross types in the same stream, and between warm- and cold-endemic cross types in different streams. Model terms followed logically from the comparative level: Degreedays (D°) was used as a predictor when comparing across tributaries to account for temperature differences, while days post fertilization (DPF) was used when comparing within tributaries, as well as either tributary or cross type. Only the data relevant to each comparative level was included in the model. Interaction effects were included in the models, because we wanted to compare the slopes of individual regressions as measures of growth. Growth is allometric: when continuous predictors are log-transformed, general linear models gave a very good fit for length and eye diameter. These models are on the form:

$$Y = \ln x + z + \ln x \times z + e$$

where Y is the response variable, x is the continuous covariate, z is the factor, e is the error term. Yolk sac volume was not modelled very accurately by conventional linear models, so generalized additive models (GAMs) were constructed using the R package *mgcv* v1.3-29 (Wood 2001). These had the form:

$$Y = f_i(x)z_i + \ldots + e$$

where Y is the response variable, f is the smoother function, x is a continuous covariate multiplied by level i of factor z, so that each spline is fitted for only the data of that particular level, and e is the error term. Fitting GAM splines required some judgement; the knots parameter (k) was reduced until a curve was found that followed the data well, while being as general as possible. It was more desirable to find the overall trend rather than to model all variation. Degrees of freedom for the splines were automatically chosen accordingly by the model fitting function of the *mgcv* package.

For hatching probability, generalized linear models (GLZs, logit link) with quasibinomial errors were fitted, over the same comparative levels, and using the same predictors. Hatching models are on the form:

$$\Pr(Y) = x + z + x \times z + e$$

where $Pr(Y)$ is the response variable, x is the continuous covariate, z is the factor and e is the error term. For an overview of the comparative levels, see table 2. Further details on the models and their parameters are presented along with the results.

Missing data posed difficulties. Most of the material from V1 was unusable, so this stream was omitted from analysis altogether. Warm endemics had high mortality in K2 and V2, and were all dead in K1. Assessment of maternal effects was only possible for K2 and V2, because there were no survivors from the V1xV1 cross type in K1. K1 was not included in the cold endemic comparisons, because it had no paired counterpart. Warm endemic comparisons were only possible for V2, and even then, the data was sparse.

Table 2. Overview of analysis. A model was created for each combination of response variable and comparative level, for a total of 28 models.

Response variables: Body length, eye diameter, yolk sac volume, hatching probability		
Comparative level	**Data used**	**Predictor variables**
Paternal effects in K1	K1xK1, K1xK2, K1xV1 in K1	Age in days, cross type
Paternal effects in K2	K2xK2, K2xK1, K2xV2 in K2	Age in days, cross type
Paternal effects in V2	V2xV2, V2xV1, V2xK2 in V2	Age in days, cross type
Maternal effects in K2	V2xK2, V2xV2 in K2	Age in days, cross type
Maternal effects in V2	K2xK2, K2xV2 in V2	Age in days, cross type
Transplanted cold endemics	K2xK2 in K2, V2	Degree days, tributary
Transplanted warm endemics	V2xV2 in K2, V2	Degree days, tributary

Model coefficients were obtained by fitting the model two times, shifting the baseline level of treatment contrasts. Predictions were made from some of the models, at the beginning, middle and late analysis period (10, 19 and 28 DPF or 100, 160, 250 D°, respectively). These are meant mainly as points of reference for judging the magnitude of differences between factor levels. Estimated times when 50% of individuals hatched were also predicted from the models.

Some predictions were used to plot reaction norms between warm and cold endemics across both temperature regimes. Slope estimates served as measures of growth rate. Reaction norms should be segregated in the presence of genetic effects, and sloped reaction norms indicates an environmental effect. Reaction norms that are not parallel means there are genotype x environment interactions present, if they cross, the genotype x environment interaction is particularly strong (Roff 1997). If the cold genotype is superior in both environments, this indicates countergradient variation (Conover & Schultz 1995). Local adaptation is suggested if the genotypes do best in their native environment (Kawecki and Ebert 2004).

For a further measure of maternal contributions by egg weight, a linear mixed effect model was fitted (due to unbalanced data) for 10 eggs per female per tributary (K1, K2 and V2), with tributary as a fixed effect and mother as a random factor effect. V1 was omitted because there were no survivors of V1 dams.

Results

General notes on results

Plots show the modelled development trajectory in a given trait (response variable), for the given factor levels, over time (days past fertilization, DPF) or temperature sum (degree-days, D°). In general, there is a significant effect of time or temperature sum on each of the traits for all comparative levels, as should be expected. Bands corresponding to a 95% confidence interval for the regression estimate are given for each curve if available, shown as dotted lines in the same colour. Analysis results are given according to comparative level, and

separately for each trait within this level. A table of model coefficients can be found in table 10, appendix.

GAM results cannot be interpreted in the same way as parametric models. To what extent trajectories of splines are different, must be judged from the splines themselves. The relative strength of trends is possible to judge when confidence bands are shown. Some summary statistics are given for the GAM models. The statistics given for the parametric model term is equivalent to an ANOVA for this term alone. Smooth terms sum to zero internally in the model, unless predictions are made on the scale of the response variable. The t-test for a smooth term is on whether it is equal to zero.

To better illustrate the development of the traits through the analysed period, a montage is given below (fig. 4).

Figure 4. Illustration of the development of the grayling embryos and larvae. The pictures are at the same scale, and are all taken from the V2 tributary. Embryos are dissected out of their shell. *Top left* – the embryo at 8 DPF, corresponding to 68 D° in this case, and 5.25 mm in length; *top middle* – 12 DPF or 123 D° and 8.11 mm length, some pigmentation is visible in the eyes, yolk sac size seems invariant from 8 DPF; *top right* – 14 DPF or 161 D° and 10.17 mm length, embryo right before hatching, with fully pigmented eyes and some body pigmentation; *middle right* – hatched larva at 16 DPF or 195 D° and 10.37 mm long, ventral and dorsal pigmentation clearly visible; *bottom* – larvae at 25 DPF or 260 D° with yolk sac nearly absorbed, 13.88 mm long. Measurement of individuals used in the analysis start at 10 DPF, in between the two first images, but many would look like in the first image.

Egg weight

The linear mixed effect model for egg weight shows a near-significant difference in means between tributaries ($F_{2,\ 34}$=3.101; p=0.058). Mean egg weights and 95% confidence intervals are given in figure 5. The difference between K2 and the other two groups is rather large by scale (0.34 mg ~ 10%).

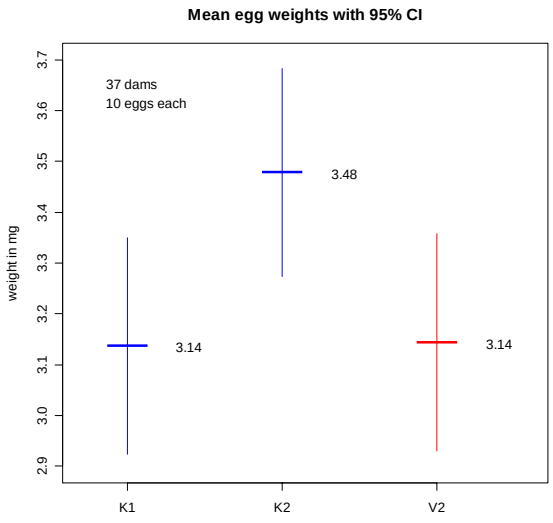


Figure 5. Mean egg weights for the three tributaries with 95% confidence intervals (vertical lines).

Cross type comparisons within tributaries – additive genetic effects

Artificial cross types with the same dam and sires from different tributaries were compared within each tributary to evaluate additive genetic effects. Cross types in this section are referred to by their paternal genotype for clarity, and the maternal genotype is the same as the tributary in which they are compared.

The paternal genotypes compared in K1 were K1, K2 and V1. For body length, there was a significant DPF by genotype interaction, and a significant genotype effect (table 3, figure 6). There was a significant genotype effect on eye diameter, but no significant difference in hatching times between genotypes. The slope for length in K2 was significantly steeper than V1 (t=2.596, 0.009), but not K1 (t=0.993, p=0.322). The length intercept for K2 significantly lower than V1 (t=-2.132, p=0.034), but not K1 (t=-0.952, p=0.342). The difference in length at 28 DPF is about 1 mm (K1: 13.16±0.27, K2: 13.89±0.20, V1: 12.93±0.36; ±95% CI). The GAM plot (figure 6) on yolk sac use showed a somewhat slower trajectory for K2 in the middle period (15-20 DPF), with confidence bands beyond the splines of the K1 and V1 genotypes, but overall, the trajectories were very similar. (Volumes at 19 DPF: K1: 41.19±4.05, K2: 46.37±3.98, V1: 40.03±7.21) Hatching times are estimated at 18.53±0.93 DPF for K1, 17.97±1.52 DPF for K2, 18.37±0.93 DPF for V1 (±95% CI).

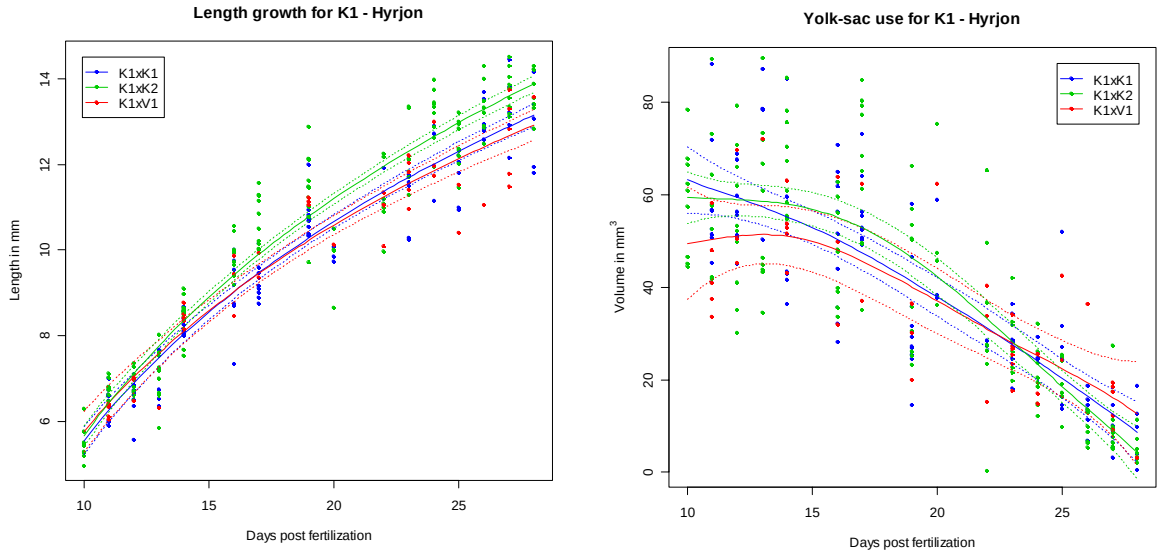


Figure 6. Model plots for same-dam cross types within the K1 tributary. Dotted lines represent 95% confidence intervals.

Table 3. ANOVA tables (type II) and additional summary statistics for same-dam models in the K1 tributary. See analysis section of materials and methods for model types. Sum sq is sum of squares, df is degrees of freedom for the corresponding model term. For model terms, ln(DPF) is log-transformed days post fertilization, genotype is a factor with levels K1xK1, K1xK2 and K1xV1, the cross types used in the models. s(DPF) is a GAM smooth term for the given factor level of the factor in the first line. Significant results, with $p < \alpha = 0.05$ are marked red. R^2 for logistic regression is calculated as R^2_{SS} = 1-SSE/SST (Mittlböck and Schemper 1996)

Model type	Response variable	Model term	Sum sq	Df	F-value	P	Adj. R^2
GLM	Length	ln(DPF)	1700.87	1	3172.78	<0.001	0.92
		Genotype	16.32	2	15.22	<0.001	
		ln(DPF)*genotype	4.51	2	4.20	0.015	
		Residuals	146.89	274			
GLM	Eye diameter	ln(DPF)	5.509	1	1148.84	<0.001	0.80
		Genotype	0.050	2	5.23	0.005	
		ln(DPF)*genotype	0.026	2	2.66	0.071	
		Residuals	1.314	274			
GAM	Yolk sac volume	Genotype		2	0.865	0.422	0.68
		s(DPF)*K1		2.028	35.02	<0.001	
		s(DPF)*K2		2.818	59.54	<0.001	
		s(DPF)*V1		2.730	9.09	<0.001	
GLZ	Pr(Hatching)	DPF	290.551	1	741.23	<0.001	0.73
		Genotype	0.378	2	0.48	0.618	
		DPF*genotype	0.681	2	0.86	0.421	
		Residuals	107.404	274			

The paternal genotypes compared in K2 were K2, K1 and V2. There was a significant effect of genotype on length (table 4), although small. Differences in intercept were insignificant. There were no significant DPF by genotype interactions for length or eye diameter. The GAM plot (figure 7) showed a seemingly significant higher volume and a following steeper decline in yolk sac volume for the V2 genotype in the early-middle period (about 12-18 DPF). Hatching times did not differ between cross types. All individuals all hatched the same day, at 17 DPF.

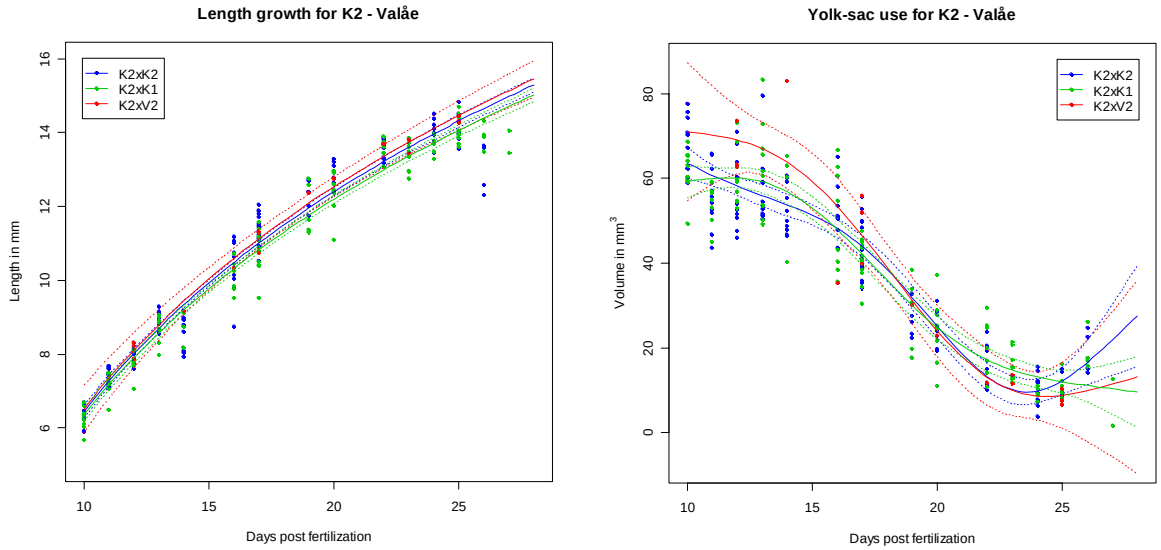


Figure 7. Model plots for same-dam cross types within the K2 tributary. Dotted lines represent 95% confidence intervals.

Table 4. ANOVA tables (type II) and additional summary statistics for same-dam models in the K2 tributary. Genotype is a factor with levels K2xK2, K2xK1 and K2xV2. See table 3 for an explanation of other elements. Hatching probability is omitted, because all cross types hatched on the same day.

Model type	Response variable	Model term	Sum sq	Df	F-value	P	Adj. R^2
GLM	Length	ln(DPF)	1654.87	1	6050.63	<0.001	0.96
		Genotype	2.49	2	4.55	0.012	
		ln(DPF)*genotype	0.24	2	0.44	0.647	
		Residuals	66.46	243			
GLM	Eye diameter	ln(DPF)	5.622	1	2063.38	<0.001	0.89
		Genotype	0.013	2	2.38	0.095	
		ln(DPF)*Genotype	0.001	2	0.26	0.768	
		Residuals	0.662	243			
GAM	Yolk sac volume	Genotype		2	1.512	0.223	0.88
		s(DPF)*K2		3.903	211.81	<0.001	
		s(DPF)*K1		3.673	210.04	<0.001	
		s(DPF)*V2		2.888	57.77	<0.001	

In V2, the paternal genotypes compared were V2, V1 and K2. There was a significant DPF by genotype interaction effect for length (table 5, figure 8). There was also a significant genotype effect on hatching. The genotype effect on eye diameter bordered on significance (p=0.051). However, there were only six observations of the V2 genotype and five for V1, so there is little power to these results. The GAM splines (figure 8) for V2 and V1 are unlikely to describe the actual trajectory (given the trajectories observed in other contexts). Because of few observations, it was also difficult to pinpoint the time of hatching for the V2 genotype. Hatching happened somewhere between 10 (last observations, unhatched) and 15 DPF (all hatched). V1 had one unhatched observation at 15 DPF and one hatched at 16 DPF. All K2 individuals hatched at 15 DPF. Thus, the genotype effect on hatching is as uncertain as other effects here.

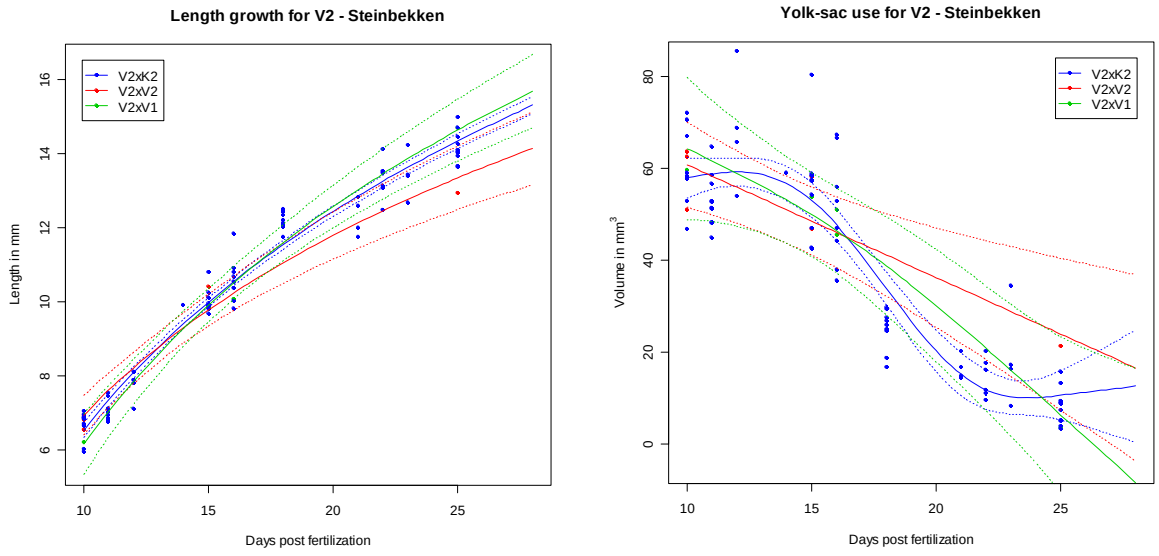


Figure 8. Model plots for same-dam cross types within the V2 tributary. Dotted lines represent 95% confidence intervals.

Table 5. ANOVA tables (type II) and additional summary statistics for same-dam models in the V2 tributary. Genotype is a factor with levels V2xV2, V2xV1 and V2xK2. See table 3 for an explanation of other elements.

Model type	Response variable	Model term	Sum sq	Df	F-value	P	Adj. R^2
GLM	Length	ln(DPF)	614.09	1	2411.73	<0.001	0.97
		Genotype	0.02	2	0.04	0.959	
		ln(DPF)*genotype	1.74	2	3.41	0.038	
		Residuals	20.62	81			
GLM	Eye diameter	ln(DPF)	2.494	1	403.26	<0.001	0.83
		Genotype	0.038	2	3.11	0.050	
		ln(DPF)*genotype	0.019	2	1.55	0.218	
		Residuals	0.501	81			
GAM	Yolk sac volume	Genotype		2	0.777	0.463	0.83
		s(DPF)*V2		1.000	12.41	<0.001	
		s(DPF)*V1		1.459	8.01	<0.001	
		s(DPF)*K2		2.947	122.22	<0.001	
GLZ	Pr(Hatching)	DPF	102.64	1	831.40	<0.001	0.91
		Genotype	3.86	2	15.65	<0.001	
		DPF*genotype	~0	2	~0	1	
		Residuals	10.00	81			

Cross type comparisons within tributaries – maternal effects

Cross types with the same sire and dams from different tributaries were compared to assess maternal effects. Sires were from the opposite temperature regime. This comparison was only done for the K2 and V2 tributaries because there were no surviving V1xV1 individuals in the K1 tributary, and no data from the V1 tributary. Cross types in this section are referred to by their maternal genotype.

In K2, comparing cross types with K2 and V2 dams, there were no significant interaction effects, but there were significant cross type effects on length, eye diameter and hatching probability (table 6, figure 9b). (Lengths at 28 DPF: K2: 15.45±0.35, V2: 14.83±0.38; ±95% CI). There was some difference in the modelled yolk sac volume trajectories (figure 9a), but the mean effect would be very similar. All K2 individuals hatched the same day, at 17 DPF. Hatching for the V2 cross type happened somewhere between 14 (last observations, unhatched) and 17 DPF (all hatched).

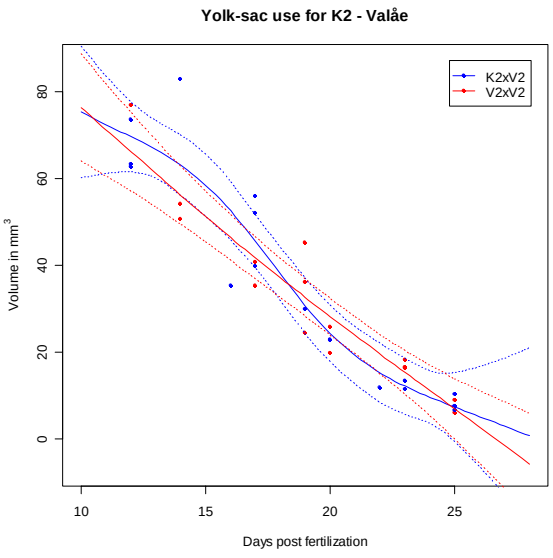


Figure 9a. GAM plot for same-sire cross types within the K2 tributary. Dotted lines represent 95% confidence intervals.

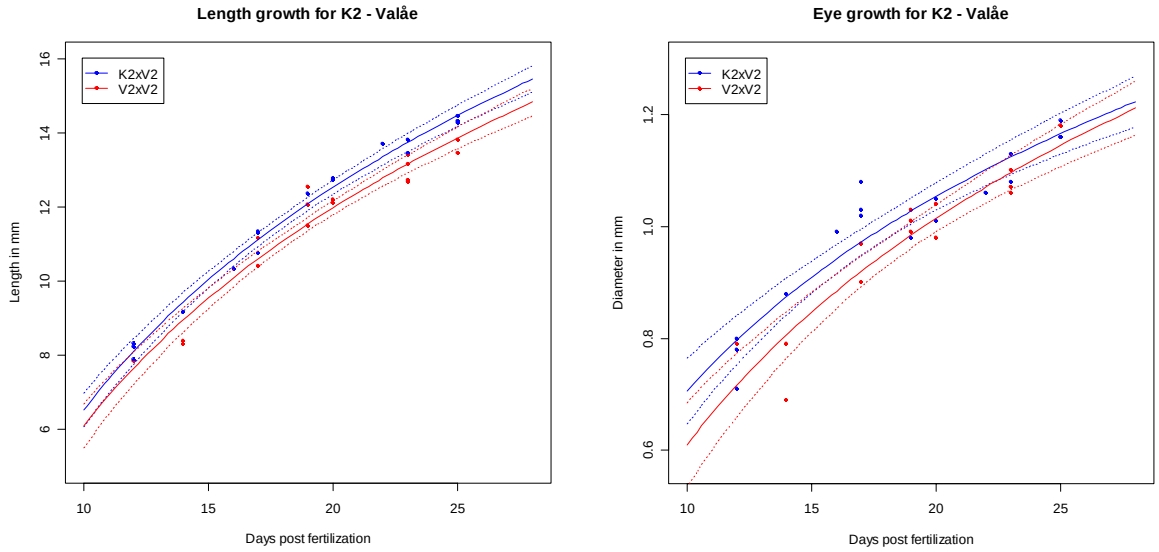
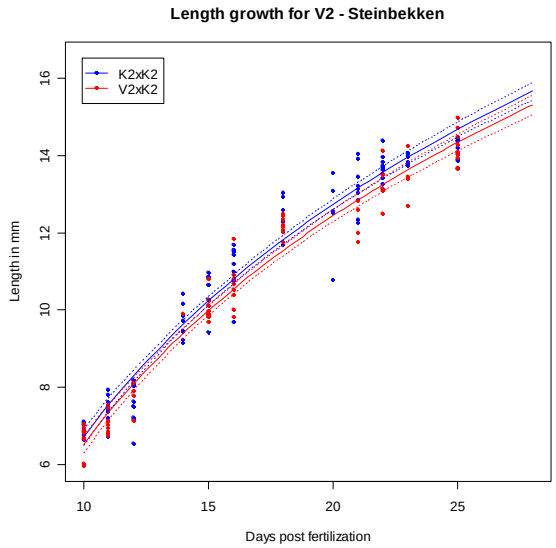


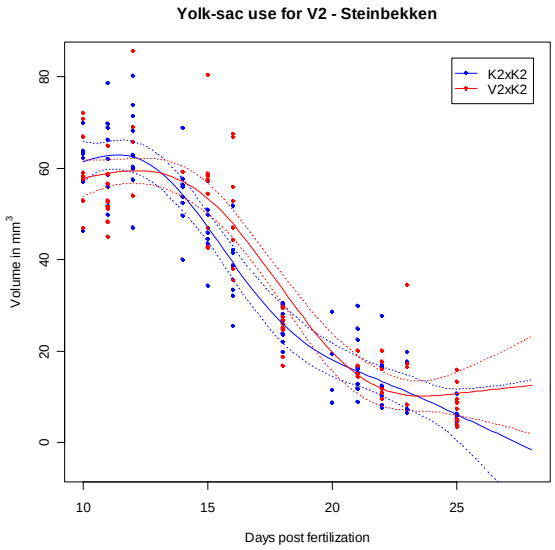
Figure 9b. Model plots for same-sire cross types within the K2 tributary. Dotted lines represent 95% confidence intervals.

Table 6. ANOVA tables (type II) and additional summary statistics for same-sire models in the K2 tributary. Cross type is a factor with levels K2xV2 and V2xV2. See table 3 for an explanation of other elements.

Model type	Response variable	Model term	Sum sq	Df	F-value	P	Adj. R^2
GLM	Length	ln(DPF)	133.69	1	949.15	<0.001	0.97
		Cross type	2.40	1	17.05	<0.001	
		ln(DPF)*cross	0.02	1	0.11	0.738	
		Residuals	4.06	29			
GLM	Eye diameter	ln(DPF)	0.519	1	224.71	<0.001	0.88
		Cross type	0.016	1	6.94	0.013	
		ln(DPF)*cross	0.003	1	1.25	0.274	
		Residuals	0.067	29			
GAM	Yolk sac volume	Cross type		1	0.032	0.860	0.89
		s(DPF)*K2		2.896	40.46	<0.001	
		s(DPF)*V2		1.322	29.83	<0.001	
GLZ	Pr(Hatching)	DPF	34.66	1	335.03	<0.001	0.93
		Cross type	2.91	1	28.14	<0.001	
		DPF*cross	~0	1	~0	1	
		Residuals	3.00	29			

In V2, comparing cross types from K2 and V2 dams, there was a significant effect of cross type on length and eye diameter (table 7, figure 10). There were no interaction effects for length, eye diameter or hatching probability. (Lengths at 28 DPF: K2: 15.66±0.23, V2:15.30±0.26; ±95% CI). According to the GAM plot (figure 10), the V2 and K2 cross types had slightly different yolk sac use trajectories. V2 was a bit delayed in its yolk absorption, compared to K2. K2 also starts out with a slightly larger yolk sac volume. All individuals for both cross types hatched at 15 DPF.





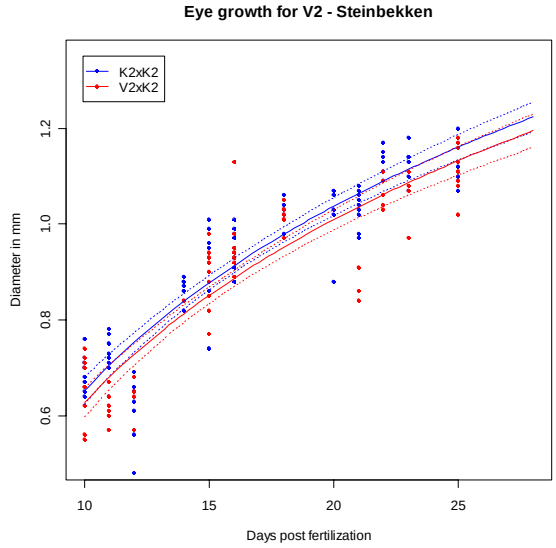


Figure 10 (above and left). Model plots for same-sire cross types within the V2 tributary. Dotted lines represent 95% confidence intervals.

Table 7 (below). ANOVA tables (type II) and additional summary statistics for same-sire models in the V2 tributary. Cross type is a factor with levels K2xK2 and V2xK2. See table 3 for an explanation of other elements. Hatching probability is omitted, because all individuals hatched the same day.

Model type	Response variable	Model term	Sum sq	Df	F-value	P	Adj. R^2
GLM	Length	ln(DPF)	1218.75	1	3951.88	<0.001	0.96
		Cross type	2.92	1	9.47	0.002	
		ln(DPF)*cross	0.12	1	0.38	0.540	
		Residuals	53.35	173			
GLM	Eye diameter	ln(DPF)	5.051	1	877.90	<0.001	0.83
		Cross type	0.031	1	5.33	0.022	
		ln(DPF)*cross	~0	1	0.01	0.910	
		Residuals	0.995	173			
GAM	Yolk sac volume	Cross type		1	2.588	0.110	0.88
		s(DPF)*K2		4.675	108.2	<0.001	
		s(DPF)*V2		3.876	127.0	<0.001	

Comparisons between transplanted cold and warm endemics

To assess environmental effects and genotype x environment interactions, the cold endemic K2xK2 cross type was compared between the V2 and K2 tributaries. K1xK1 in K1 was omitted from analysis because there was no paired stream to compare responses. The few surviving warm endemics (V2xV2) were also compared between the V2 and K2 tributaries. With degree-days as a covariate, the effect of temperature is essentially removed.

For the K2 genotype, there were significant temperature sum (D°) by tributary interactions and tributary effects for both length and eye diameter (table 8, figure 11) There was also a significant tributary effect on hatching probability. The GAM plot (figure 11) showed slightly different trajectories in the 100-170 D° period, and yolk sac absorption was a bit delayed in V2. All hatching occurred at 166.2 D° in K2, corresponding to 17 DPF for this tributary. In V2, all hatching occurred at 161.1 D°, corresponding to 15 DPF for this tributary, a difference of about 5 D°.

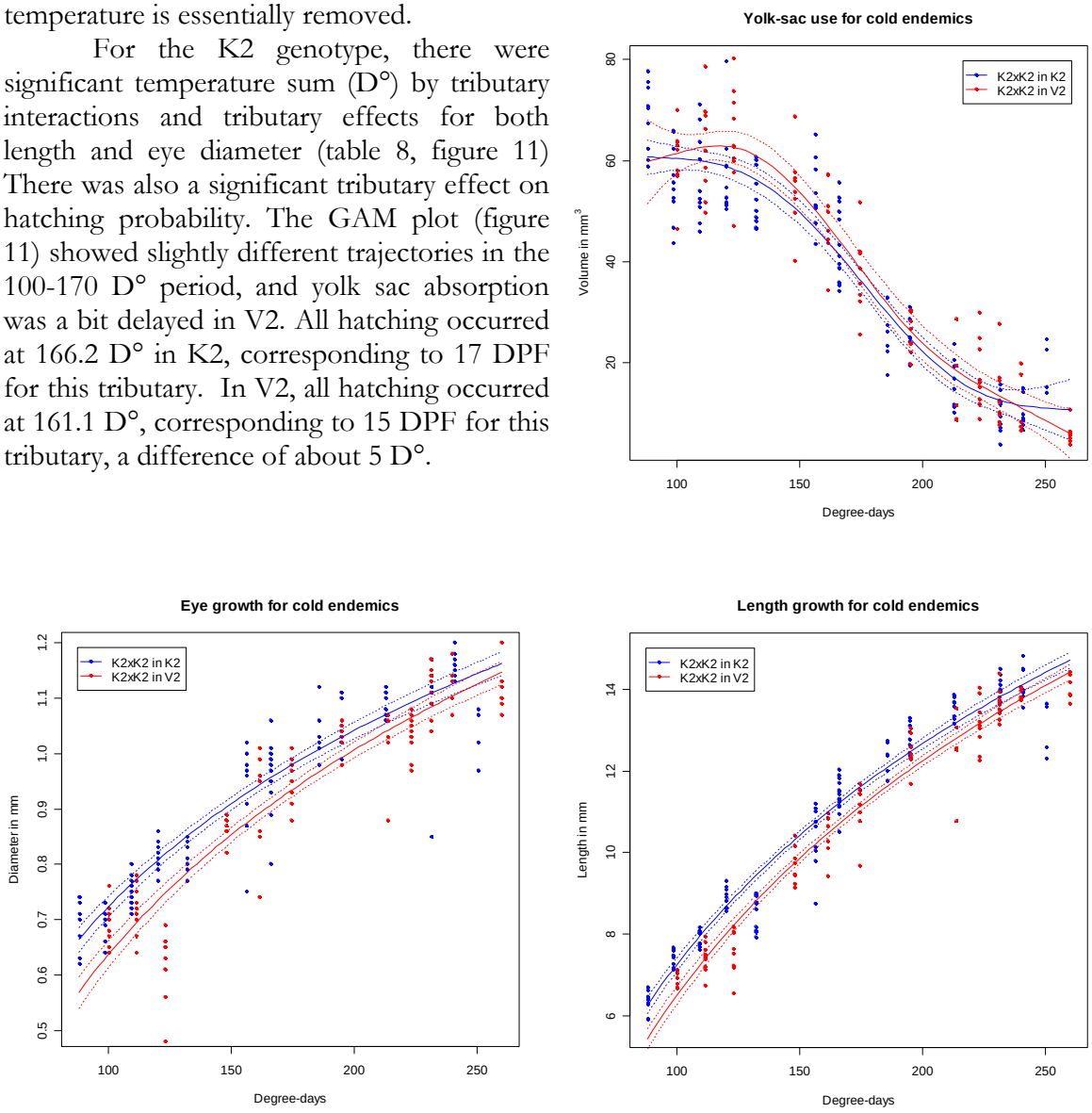


Figure 11. Model plots for the K2 genotype compared between the K2 and V2 tributaries. Dotted lines represent 95% confidence intervals.

Table 8. ANOVA tables (type II) and additional summary statistics for fitted models on cold endemics. Trib is a factor with levels K2 and V2, the two tributaries compared. See table 3 for an explanation of other elements.

Model type	Response variable	Model term	Sum sq	Df	F-value	P	Adj. R^2
GLM	Length	ln(D°)	1469.32	1	4569.58	<0.001	0.95
		Trib	14.78	1	45.97	<0.001	
		ln(D°)*Trib	1.29	1	4.00	0.047	
		Residuals	69.45	216			
GLM	Eye diameter	ln(D°)	5.485	1	1350.79	<0.001	0.84
		Trib	0.138	1	34.06	<0.001	
		ln(D°)*Trib	0.030	1	7.34	0.007	
		Residuals	0.877	216			
GAM	Yolk sac volume	Trib		1	3.157	0.077	0.89
		s(D°)*K2		2.949	274.91	<0.001	
		s(D°)*V2		4.399	97.65	<0.001	
GLZ	Pr(Hatching)	D°	267.31	1	2405.8	<0.001	0.92
		Trib	8.99	1	80.9	<0.001	
		D°*Trib	~0	1	~0	1	
		Residuals	24.00	216			

The V2 genotype was compared between the K2 and V2 tributaries. Very few observations formed the basis of this analysis, giving it little power. Significant temperature sum by tributary interactions were found for both length and eye diameter (table 9). There was also a significant effect of tributary on length. The GAM plot (figure 12a) showed a slower yolk sac use trajectory in V2 compared to K2. Examining the other plots as well (figure 12b), it is clear that the endpoint of each curve for the V2 tributary was defined by only one observation. More observations would undoubtedly have changed the trajectories of these curves. Interestingly, several observations to which the V2 curves were fitted fell well beyond the residual variation of K2 observations. Hatching occurred somewhere between 14 (last observation, none hatched) and 17 DPF (all hatched) for individuals in K2, corresponding to 132.3 and 166.2 D°, respectively. In V2, hatching happened somewhere between 10 (none hatched) and 15 DPF (all hatched), corresponding to 100.5 and 161.7 D°, respectively.

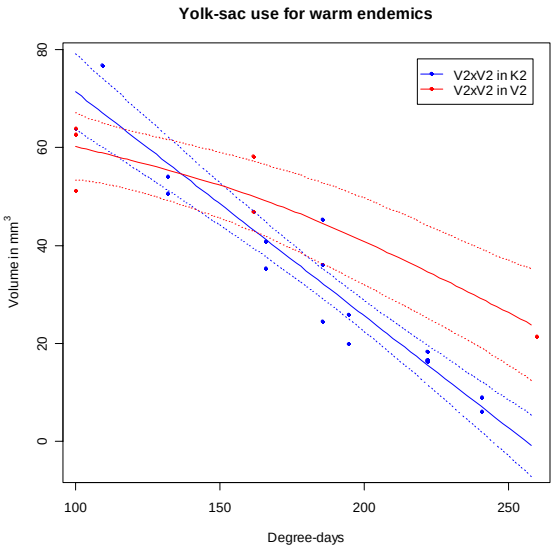


Figure 12a. GAM plot for the V2 genotype compared between the K2 and V2 tributaries. Dotted lines represent 95% confidence intervals.

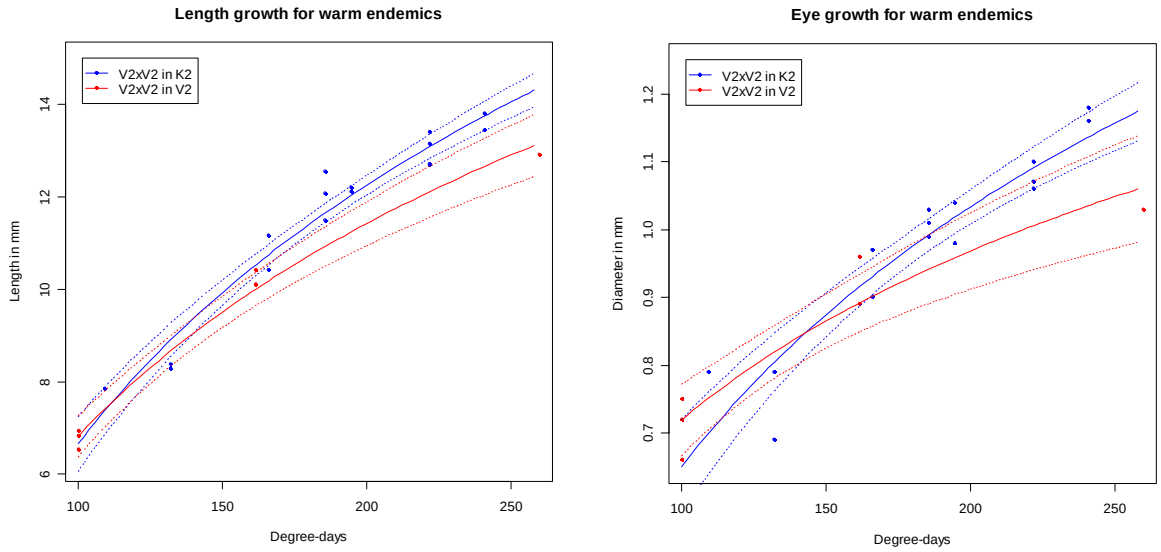


Figure 12b. Model plots for the V2 genotype compared between the K2 and V2 tributaries. Dotted lines represent 95% confidence intervals.

Table 9. ANOVA tables (type II) and additional summary statistics for fitted models on warm endemics. Trib is a factor with levels K2 and V2, the two tributaries compared. See table 3 for an explanation of other elements. Hatching probability is omitted because analysis was difficult and redundant with so few observations.

Model type	Response variable	Model term	Sum sq	Df	F-value	P	Adj. R^2
GLM	Length	ln(D°)	84.941	1	501.1493	<0.001	0.97
		Trib	0.886	1	5.2253	0.034	
		ln(D°)*Trib	0.801	1	4.7230	0.043	
		Residuals	3.051	18			
GLM	Eye diameter	ln(D°)	0.3286	1	139.10	<0.001	0.90
		Trib	0.0015	1	0.64	0.435	
		ln(D°)*Trib	0.0145	1	6.15	0.023	
		Residuals	0.0425	18			
GAM	Yolk sac volume	Trib		1	6.968	0.017	0.90
		s(D°)*V2		1.000	127.02	<0.001	
		s(D°)*K2		1.513	13.88	<0.001	

Predictions were made from the cold and warm endemic models at 100 (early period), 160 (assumed hatching) and 250 (late period) D°. Reaction norms were plotted based on these predictions between native and transplanted environments (figure 13). Sloping reaction norms, apparent in all plots, indicate environmental effects. Segregation of the reaction norms indicates genetic and/or maternal effects, and is apparent at least for length at 160 and 250 D°. The reaction norms for length at 100 D° seem to indicate a strong genotype x environment interaction; the K2 genotype has a negative environmental response, while the V2 genotype has a positive environmental response. Confidence intervals are rather large for V2 transplants. There is no apparent genotype x environment interaction for length around the time of hatching (160 D°). A genotype x environment interaction is again present at 250 D°, with a negative environmental response for both genotypes – both become larger in the K2 tributary. There is little difference in yolk sac volume for the K2 genotype at hatching. There could be a difference on the scale of 5 mm^3 for the V2 genotype, but with a large overlap in confidence intervals.

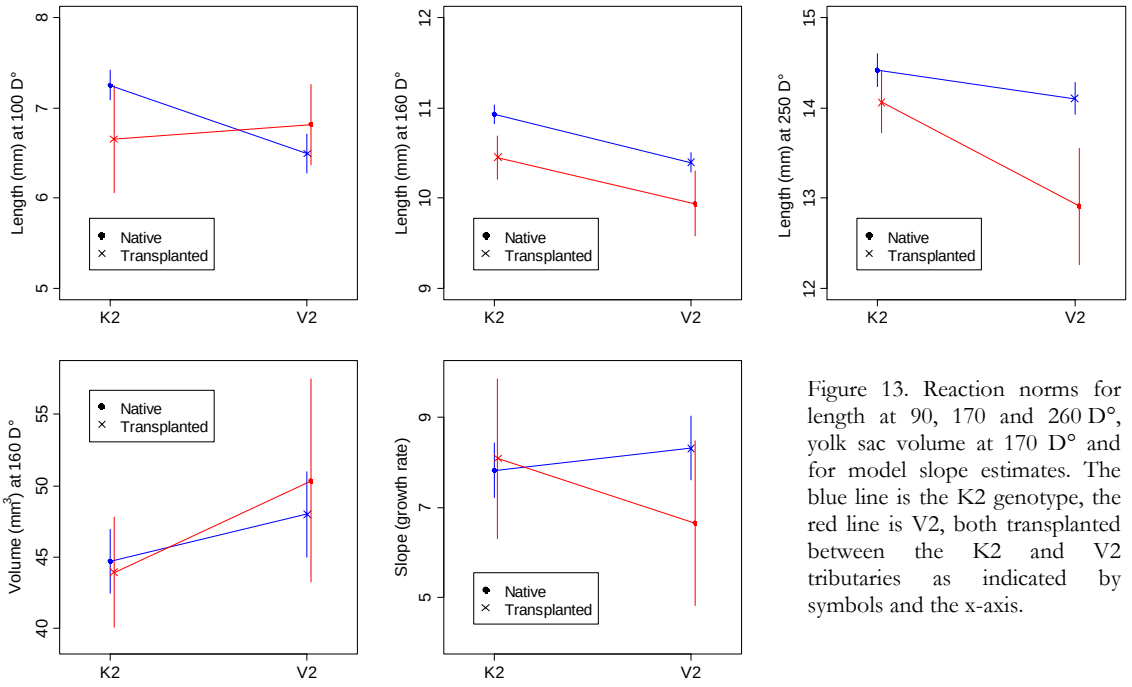


Figure 13. Reaction norms for length at 90, 170 and 260 D°, yolk sac volume at 170 D° and for model slope estimates. The blue line is the K2 genotype, the red line is V2, both transplanted between the K2 and V2 tributaries as indicated by symbols and the x-axis.

Discussion

I found evidence of significant additive genetic effects on growth rate. The relative differences between K1 and K2 are not consistent between tributaries, so there seems to be a component of genotype x environment interaction; the responses of the genotypes differ between environments. It is not consistent with local adaptation, in which the genotypes would do best in their local environment. There seems to be weak genetic effects on yolk sac use, and no significant differences in hatching time in due to genetic effects, where data were available.

There are differences in mean egg size between dams used in crosses, although just above the significance level – K2 dams had larger eggs than K1 and V2 dams. There are also significant maternal effects on body length and eye diameter. Results are consistent between the K2 and V2 streams – K2 embryos/larvae are larger in both. The absence of significant genetic effects between K2 and V2 where sufficient data were available, suggests the size displacement observed is attributable to the difference in egg weight. Bigger eggs give bigger embryos/larvae. Concerning yolk sac absorption, the differences are small. Hatching did not differ significantly due to maternal effects, where sufficient data is available.

The cold-warm transplant comparison shows differences in growth between environments for each genotype. Environmental effects even after adjusting for temperature sum indicates that there are large sources of variation that are not accounted for. Volume differences at hatching are negligible. Hatching differences due to environment is significant for the cold endemic, but biologically a difference of 5 D° is not very large; it corresponds to about half a day at this stage. Between cold and warm tributaries, reaction norms reveal that there are some genotype differences, but the dominance of K2 is not consistent. However, I have shown significant maternal effects, and the evidence on genotype effects between the cold and warm genotype either had very small sample size, or was small and insignificant. The observed difference can again be due to maternal effects. Signatures of genotype x environment interaction between the cold and warm endemics are hard to explain, as the

source of environmental variation is cryptic. They are for the most part inconsistent with local adaptation (the cold genotype would have favoured cold environment and vice versa).

In sum, the additive genotype effects found cannot be separated from genotype x environment interaction, but the presence of significant additive genetic variation confirm that there is room for adaptation in these traits. Maternal effects are likely, and suggest the possibility of an adaptive increase in egg size. Maternal effects may also explain the "genotype" difference between cold and warm endemics. Countergradient growth could also have been due to maternal effects, but K1 has small eggs, and this does not consistently support the countergradient adaptation hypothesis.

Additive genetic variation and adaptive potential

My results indicate there are additive genetic effects on growth rate. The grayling in Lesjaskogsvatn have previously been shown to retain adaptive potential in spite of a severe bottleneck, small initial population size and a current metapopulation structure with some gene flow (Haugen and Vøllestad 2000; Koskinen et al 2002; Gregersen et al 2008; Kavanagh et al 2008). As a tentative explanation of why this is so, I will review some of the theory available.

Willi et al (2006) summarize the effects of bottlenecks and population size on the adaptive potential in small populations. According to neutral theory, bottlenecks should reduce additive genetic variation (V_A) through genetic drift, but the picture is complicated by epistasis and dominance effects. These remaining portions of total genetic variance ($V_G = V_A + V_D + V_I$, where V_I is variation due interactions between loci, or epistatic variation, and V_D variance due to dominance effects) are often ignored, at least in the case of epistatic interaction, because they greatly complicate quantitative genetic theory (Roff 1997). Epistasis and dominance can increase V_A, at least temporarily (Willi et al 2006). Successful increase in genetic variance and potential for adaptation of the Lesjaskogsvatn grayling, despite minimal initial conditions, may stem from such an effect. This is consistent with the short time since colonization of the system.

Gene flow can limit the potential for adaptation by homogenizing demes, but it can also increase genetic variance towards the level of the metapopulation as a whole, if the level of gene flow is low (Willi et al 2006). Some gene flow, then, can be helpful to adaptation. The homing behaviour of grayling (Kristiansen and Døving 1996) will limit gene flow to some extent, but the Lesjaskogsvatn demes do not show complete isolation (Barson et al 2008). If sufficient selection pressures are present, adaptation to local environmental conditions can take place in the presence of limted gene flow (Kawecki and Ebert 2004).

Roff (1997) supplies the basics on heritability. Narrow-sense heritability (h^2) has been frequently used as a measure of the capacity for a given trait to respond to selection, because of its place in the *breeders equation* $R = h^2S$, where S is the selection differential, the difference in a population mean before and after the selection in a singe generation. Heritability itself is defined as $h^2 = V_A/V_P$, where V_A is additive genetic variance, and V_P is phenotypic variance; h^2 then, is the proportion of phenotypic variance explained by additive genetic variance. The usefulness of h^2 alone as a measure of the capacity to respond to selection is limited, because response also depends on the selection differential, S. The selection differential is a measure of how strong selection acting on a given trait. The heritability of life history traits is on average low.

Consequently, selection would need to be very strong to drive adaptation in life history traits under the conditions in Lesjaskogsvatn. Indeed, Koskinen et al (2002) showed that directional selection was the dominant diversifying agent between Lesjaskogsvatn and nearby lakes – the values of standardized selection differentials between them were very

high. Adaptive potential in Lesjaskogsvatn may have been conserved – and indeed enhanced – by a combination of epistasis or dominance effects on additive genetic variation, limited gene flow between demes, and strong directional selection.

The importance of maternal contributions

The observed differences in mean egg size between the dams used in crosses are likely to explain the size differences in the analysis of maternal effects. Larger eggs are known to result in larger offspring in salmonids (e.g. Kamler 1992).

The presence of maternal effects and differences in egg weight suggest there is at least opportunity of adaptive increase in egg size. Gregersen et al (2008) found evidence of divergence in egg size, adjusted for the size of the mother, between small, warm tributaries and large, cold tributaries in Lesjaskogsvatn. Larger eggs in warm streams were thought to be the result of high temperatures reinforcing the selective advantage of large eggs in the presence of density-dependent fry interactions. Although not comparable to the present findings on maternal effects, it illustrates that there is potential for adaptation in egg size in Lesjaskogsvatn. Such adaptation, of course, requires that egg size is under genetic control. A lot of evidence on genetic control of egg size exists, but there is also evidence for a trade-off with fecundity (Roff 1992), including in salmonids.

Are bigger offspring more fit? Starvation and predation are widely recognized as the two most important causes of mortality in the early life stages of fish, and they are both size dependent – larger juveniles are less susceptible (e.g. Kamler 2005). However, many effects can complicate the picture. The fitness advantage of being large can be density- and environment dependent. Einum and Fleming (1999) suggested that the fitness advantage of larger juveniles in brown trout (*Salmo trutta*) is large in a high-density environment, but smaller in a low-density environment. A connection to starvation mortality was made, as high-density environments have greater competition for resources. This has consequences for the size-fecundity trade-off: In a low-density environment, females should produce more and smaller eggs to optimize fitness. Einum (2001) later found that positive effects of egg size on offspring fitness in Atlantic salmon (*Salmo salar*) were apparent even under strong food-limitation, suggesting that the negative effect of being large is related to fry interactions at high densities, not to food availability itself. Gregersen et al (2008) confirmed this same effect in Lesjaskogsvatn. The size-dependent mortality from predation is related to the size of predators; large predators can eat both small and large prey, while small predators can only eat the smaller prey – also, small predators are more numerous than small ones (Last 1980, reviewed by Kamler 2005). As an opposing effect, larger juveniles may be more exposed to predation when there is lack of shelter (Finstad et al 2007). In summary, selection on juvenile size can influence the evolution of maternal traits in several ways. Bigger offspring are in general more fit, at least in the absence of high fry interaction and lack of shelter. If there is sufficient selection for large eggs, and consequently larger offspring, egg size will increase to an optimum balance with fecundity.

Countergradient variation and the disparity between previous and present findings

Countergradient variation is the specific case of genetic-environmental covariance where genetic and environmental influences on phenotype reinforce one another (Conover and Schultz 1995). Examples of countergradient variation in fish are found in Atlantic silversides (Conover and Present 1990) and pumpkinseed sunfish (Arendt and Wilson 1999), among others. My results do not show a pattern consistent with countergradient variation, although the results from warm endemics may be unrepresentative due to sample size.

Prior evidence for countergradient variation in Lesjaskogsvatn (Kavanagh et al 2008) comes from a controlled common garden lab study. Growth rate and yolk absorption rate were higher in cold endemics. If we assume that the original assertions hold, variables that are not controlled or considered in this study may explain the pattern evident here. Indeed, I have shown that there are large unexplained environmental influences, the different responses and sloping reaction norms of cold and warm genotypes in my results suggest that these traits are highly influenced by variables other than temperature. Such variables may include e.g. location in the stream, gravel qualities, oxygen content in the water, water chemistry, stream discharge, and diel temperature variations. Most such variables are constant in a laboratory setting; hence, laboratory results are misleading as demonstrations of the real-life phenotypic expression of said traits. More studies should be conducted in the field, if we are to understand them.

If further evidence of countergradient variation is found, we lack several details to explain it. Increased growth is considered to have strong positive association with fitness, but life history theory predicts that there may be trade-offs between growth and survival (Stearns 1992). If there is capacity of a growth increase, why do we not see it universally? Such a trade-off was not found by Kavanagh et al (2008). It is not given that increased growth is an adaptation at all. For adaptation to be proven, increased growth must be shown to increase fitness. We currently have no data on whether such a growth rate persists, on possible trade-offs, or its long-term fitness.

Notes on the analysis and further future considerations

I recognize that the extent of my analysis is by no means complete. The rather simple approach I ended up with was designed to answer the questions posed. There may still be a lot of information to be salvaged from this data, but further exploration of the field of quantitative genetics and its methods would be necessary. Analysing traits by multivariate methods could help find correlations that are not accounted for by a discrete approach. Incorporation of additional model terms could have explained residual environmental effects, although I found none that did. Other model types could have been employed for yolk sac modelling – piecewise regression, polynomial regression and non-linear parametric regression were explored with limited success. They could have given less vague answers.

For the consideration of future studies similar to this one, it would be interesting to extend the scope. A small subset of the spawning tributaries has been examined in previous or current studies of this system. Different sets of them would need to be included to generalize our findings. A more complete transplant between a limited number of tributaries would be manageable, if it was well planned. This would give us even more data if all went well, and would buffer against unforeseen consequences. We would also have an increased possibility of separating interaction effects from the effects of single variables. To secure the investment in work hours at any scale, great care should be taken in handling and storage of the gametes. Care must be taken when stripping the fish of gametes, good refrigeration is essential, and careful, efficient handling in the vulnerable period right after fertilization. Any measurements of environmental variables that can reasonably be taken can help us understand residual environmental variance.

Acknowledgements

I would like to thank my supervisor L. Asbjørn Vøllestad for all his knowledge and extensive guidance, for literally always having his door open, for being accessible, friendly and informal. I also thank co-supervisor Thrond O. Haugen for guiding the experiment design, for organizing and directing much of the field work, for his knowledge on the grayling and study system, and tips and pointers on the analysis. Finn Gregersen had an invaluable role in organizing and contributing to the fieldwork. Erica H. Leder, Stephanie M. Carlson and Kim Magnus Bærum participated in the fieldwork; I hope my help to them was as valuable as theirs was to me. Leif Christian Stige and Eric Edeline at CEES gave some useful pointers on the technical side of the analysis. Nicola Barson seeded an interest in topics that proved useful in this thesis, and will be useful in the future.

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Appendix

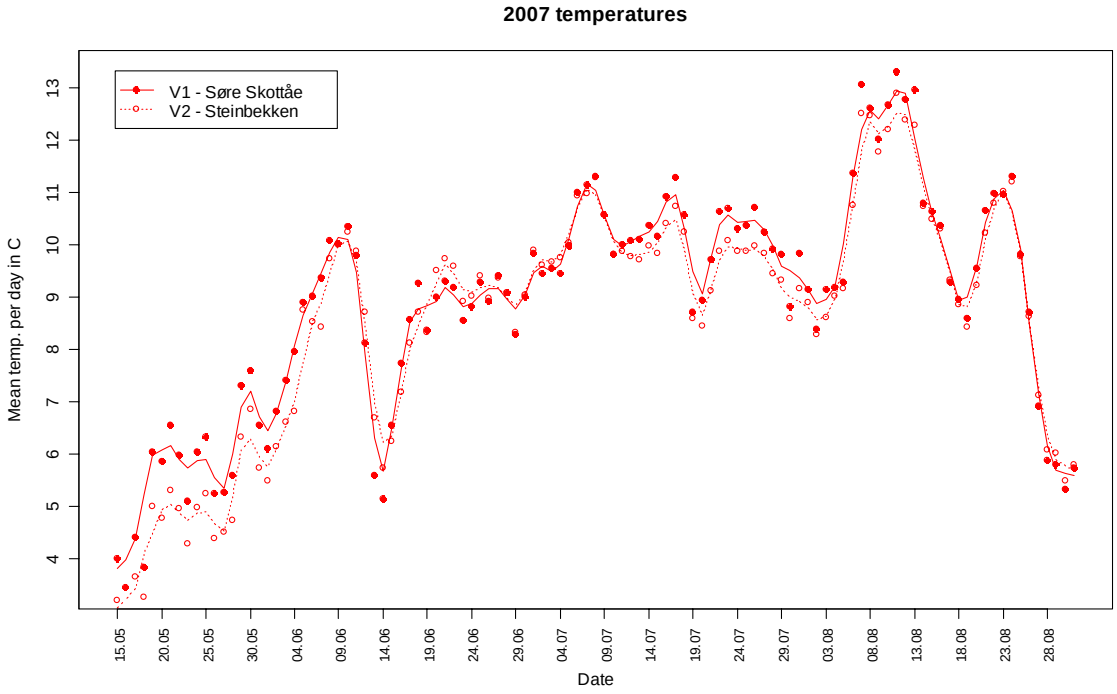

Figure 14. Temperatures in the V1 and V2 streams followed each other very closely in 2007. The data is highly correlated; r=0.98, n=109.

Table 10 (contd. next page). Model coefficient estimates from general linear models. Factor is according to the comparative level. For paternal comparisons, dam is from the stream in comparative level, sire is the factor. For maternal comparisons, sire is from the stream opposite the comparative level, dam is the factor. For cold comparisons, the cross type is K2xK2, factor is the stream of residence. For warm comparisons, the cross type is V2xV2, factor is the stream of residence.

Comparative level	Trait	Coefficient	Factor	Estimate	SE
Paternal, K1	Length	Slope	K1	7.39	0.25
			K2	7.99	0.18
			V1	6.96	0.35
		Intercept	K1	-11.47	0.73
			K2	-12.75	0.53
			V1	-10.26	1.04
	Eye diameter	Slope	K1	0.433	0.024
			K2	0.456	0.017
			V1	0.369	0.034
		Intercept	K1	-0.364	0.069
			K2	-0.191	0.098
			V1	-0.409	0.050
Paternal, K2	Length	Slope	K2	8.59	0.16
			K1	8.39	0.16
			V2	8.66	0.50
		Intercept	K2	-13.32	0.45
			K1	-12.94	0.45
			V2	-13.43	1.46
	Eye diameter	Slope	K2	0.503	0.016
			K1	0.487	0.016
			V2	0.503	0.050
		Intercept	K2	-0.481	0.045
			K1	-0.431	0.045
			V2	-0.453	0.146

Paternal, V2	Length	Slope	V2	6.99	0.60
			V1	9.25	0.76
			K2	8.51	0.18
		Intercept	V2	-9.14	1.61
			V1	-15.14	2.15
			K2	-13.06	0.51
	Eye diameter	Slope	V2	0.375	0.096
			V1	0.540	0.121
			K2	0.552	0.029
		Intercept	V2	-0.136	0.251
			V1	-0.531	0.335
			K2	-0.642	0.080
Maternal, K2	Length	Slope	K2	8.67	0.36
			V2	8.48	0.44
		Intercept	K2	-13.44	1.04
			V2	-13.63	1.28
	Eye diameter	Slope	K2	0.503	0.046
			V2	0.584	0.057
		Intercept	K2	-0.45	0.13
			V2	-0.74	0.17
Maternal, V2	Length	Slope	K2	8.68	0.19
			V2	8.51	0.20
		Intercept	K2	-13.26	0.52
			V2	-13.06	0.56
	Eye diameter	Slope	K2	0.556	0.025
			V2	0.552	0.028
		Intercept	K2	-0.63	0.070
			V2	-0.64	0.077
Cold endemics	Length	Slope	K2	7.82	0.16
			V2	8.30	0.18
		Intercept	K2	-28.78	0.79
			V2	-31.74	0.93
	Eye diameter	Slope	K2	0.46	0.018
			V2	0.53	0.020
		Intercept	K2	-1.39	0.089
			V2	-1.81	0.105
Warm endemics	Length	Slope	K2	8.08	0.46
			V2	6.64	0.47
		Intercept	K2	-30.57	2.40
			V2	-23.80	2.34
	Eye diameter	Slope	K2	0.55	0.054
			V2	0.36	0.056
		Intercept	K2	-1.89	0.283
			V2	-0.94	0.277